borders of the tubular cells. The composition of the particles and their appearance in electron microscopy would identify them as derived from the membranes. Thus, the particles may be considered to be derived from the membranes of the brush borders of the tubular cells and, in view of their constant composition, consistent behavior in centrifugation, and their unique and constant enzymatic content, we propose the term "nephrosomes" as a convenient designation.

FRANCIS BINKLEY, NEVA KING
ELIZABETH MILIKIN, REBA K. WRIGHT
C. H. O'NEAL, INA JANE WUNDRAM
Departments of Biochemistry and
Anatomy, Emory University,
Atlanta, Georgia 30322

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15. Supported by PHS research grant AM06089. Publication No. 892 of the Division of Basic Sciences.

18 July 1968

Morphine: Single-Dose Tolerance

Abstract. *Rats show a significant degree of tolerance to a second dose of morphine, with the degree of tolerance increasing the longer the delay between the two doses of morphine. To measure the morphine effect a foot-shock attenuation procedure that allowed the animal to adjust the shock intensity was used in studying delays of up to 180 days.*

A single dose of morphine sulfate (MS) in the rat results in a significant degree of tolerance to a second dose given several months later (*1*). We now report results of a study designed to determine whether this single-dose tolerance is independent of the time interval between the first and second dose.

Seven groups of experimental animals and seven groups of control animals (male, albino Holtzman; 175 to 250 g) consisted of eight animals, with the exception of one control and one experimental group that had five animals in each. On day 1, all experimental rats were given a single subcutaneous dose of 10 mg of MS (in 1 ml of saline) per kilogram of body weight and all control rats were given 1 ml of saline alone. Animals were then returned to their home cages. On day 2 (24 hours later) one group of experimental and one group of control subjects were tested on a shock attenuation procedure (*2*) after receiving 5 mg of MS per kilogram of body weight. On day 4, a second experimental and a second control group were tested after a dose of 5 mg/kg. Other groups were tested on days 8, 16, 32, and 180. (The groups tested on day 180 consisted of five experimental and five control subjects.)

To test the effects of the drug, we used a chamber (20.3 by 22.8 cm) with a paddle wheel 15.2 cm long and 7.6 cm in diameter, placed 3.8 cm above the grid floor in the shorter wall of the chamber. On a test day, animals were trained to escape from a gradually increasing shock by rotating the wheel one-quarter of a revolution. The shock intensity increased 0.02 ma every 15 seconds. When the animal turned the wheel, he terminated the shock (escaped) for 15 seconds. After 15 seconds, the shock returned, but at the next lower intensity. By this means the animal could maintain a "comfortable level" of shock intensity (Fig. 1). Animals were tested for 150 minutes after the drug was given. By means of a planimeter, the area under the time-effect curve was determined in square centimeters. The data were transformed by obtaining the square root of this area as well as the square root of the area during the 30-minute period prior to administration of the drug.

Since it was found that the score after administration of the drug was significantly correlated with the score prior to administration of the drug ($r=.53$) we obtained an adjusted difference score (*3*) that allowed further statistical computation in which the variance due to the initial level was removed. A "predicted" score for an individual animal for the period was determined by the use of the regression equation for drug scores before and

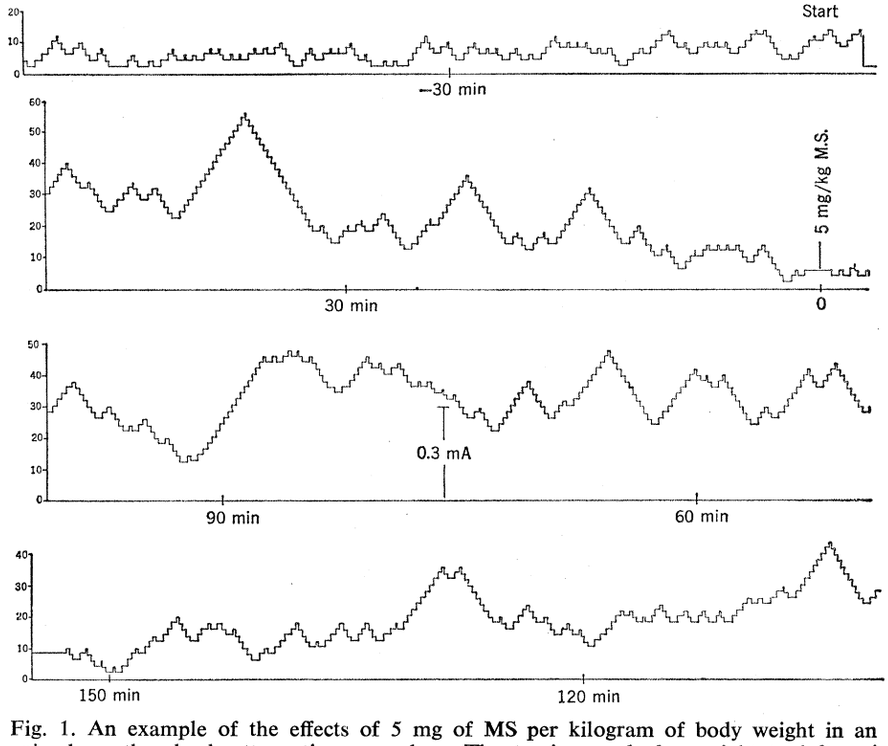

Fig. 1. An example of the effects of 5 mg of MS per kilogram of body weight in an animal on the shock attenuation procedure. The tracing reads from right to left and from top to bottom. The vertical distance is a linear representation of the shock intensity, and the horizontal distance represents time.

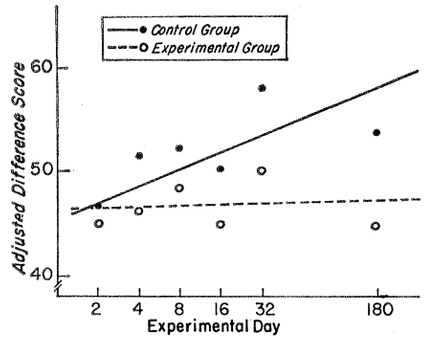

Fig. 2. Regression lines of best fit for control and experimental animals. The larger the adjusted difference score, the greater the effect of MS.

after administration. The difference between the "predicted" score and the score after drug administration was used for analysis. However, in order to avoid the use of negative scores in Fig. 2 and in Table 1, the mean predicted score was added to the mean of the adjusted difference scores. With the mean predicted score as a constant, we brought the adjusted score back to the magnitude of the square root of the area under the time-effect curve.

Figure 2 shows the mean adjusted difference score at each of the time intervals. The slopes of the experimental and control regression lines are 0.55 and 5.22, respectively. These slopes differ by means of t-test at an acceptable level of confidence ($t = 2.05$, $P < .05$). As compared to the control animals, the experimental animals had an attenuated effect to the drug, and the attenuation of the morphine effect is greater, the longer the time between the initial dose and the test dose. The control animals tested late in the experiment had a more intense reaction to the MS (5 mg/kg) than those control animals tested early (Fig. 2). This greater response over a period of time may have been due to the increase in weight of the animals. On day 1, the mean weight was 200 g, and on day 32 it was 350 g. (The weight was the same for control and experimental animals throughout the experiment.) Since the dose was in milligrams per kilogram of body weight, the heavier animals received more drug. This suggests that giving MS in doses of miligrams per kilogram does not equate animals of different weights.

Table 1. Mean adjusted difference scores for both control and experimental groups in experiment 2. On day 8, $P < .025$ (single-tail t-test) between control and experimental groups; and $P < .05$ (single-tail t-test) between the two experimental groups.

Group	Scores	
	Day 2	Day 8
Control	33.65	35.83
Experimental	35.30	29.20

In order to remove the confounding of the change in weights of the animals, we repeated the experiment for days 2 and 8. In this experiment, the weights of the animals were kept constant; that is, the animals in all groups tested were approximately the same age and weights, a condition achieved by giving the initial dose of 10 mg/kg at different times before the test day (Table 1). There was no evidence of tolerance on day 2 of the experiment, but there was marked evidence on day 8.

These results confirm the phenomenon of single-dose tolerance in the rat and indicate that, at these doses, the tolerance is not present 24 hours after the initial dose was given. In fact, the tolerance becomes more pronounced the longer the time interval between the two doses of the drug. The mechanism for this type of tolerance may be quite different from that after repeated large doses of MS.

One hypothesis that has been proffered is that this single-dose tolerance may be the result of an immune mechanism. Passive transfer experiments have suggested that this may be so (*4*). However, these experiments have not given consistent results. In fact, there is some evidence suggesting that there may be a potentiating factor in the serum of the animals previously made tolerant to MS (*5*). Thus the enigma of tolerance to this drug is not solved but only made more complicated by the results of this experiment.

CONAN KORNETSKY
GEORGE BAIN

Division of Psychiatry,
Boston University School of Medicine,
Boston, Massachusetts 02118

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15 April 1968

Nonspecific Staining: Its Control in Immunofluorescence Examination of Soil

Abstract. *Gelatin preparations were used to treat soil slides prior to addition of fluorescent antibody. Nonspecific staining was avoided, with no detectable interference to specific staining. Gelatin-rhodamine conjugates served to counterstain as well as to prevent nonspecific staining.*

Many applications of the fluorescent antibody (FA) technique are complicated by nonspecific staining reactions resulting from retention of fluorescent components of a conjugate by mechanisms other than known immunologic reactions. Nonspecific staining may interfere with the FA detection of pathogenic bacteria in host tissues in diagnostic procedures, particularly when the conjugates are undiluted or diluted only slightly (*2*). We have used the FA technique to study soil bacteria *in situ* and encountered problems due to nonspecific staining (*3*). We now report that we can control nonspecific staining in the immunofluorescence examination of soil preparations, and have applied this technique to some problems in diagnostic microbiology.

Gelatin solutions modified by partial alkaline hydrolysis prevented nonspecific staining. When the modified gelatin was conjugated to the fluorochrome dye rhodamine isothiocyanate (RhITC), the preparation provided additional desirable features as an effective counterstain. The gelatin apparently adsorbs to soil and tissue, blocking sites of nonspecific adsorption; the dye conjugated to the gelatin imparts an orange-brown background fluorescence to the soil or tissue in good contrast to the yellow-green of a fluorescein-labeled antibody.

A 2-percent aqueous solution of gelatin (*4*), adjusted to *p*H 10 to 11 with 1*N* NaOH, was autoclaved for 10 minutes at 121°C; the autoclaved solution was readjusted to the same *p*H. The gelatin was conjugated by (i) dis-